Cell Meter™ Intracellular NADH/NADPH Fluorescence Imaging Kit

Catalog number: 15290
Unit size: 100 Tests

Component	Storage	Amount
Component A: JZL1707 NAD(P)H Sensor	Freeze (<-15 °C), Minimize light exposure	1 vial (40 µL)
Component B: Assay Buffer	Freeze (<-15 °C)	1 bottle (20 mL)

OVERVIEW

The detection of intracellular dihydronicotinamide adenine dinucleotide NADH and its phosphate ester NADPH is important for disease diagnostics and drug discovery. In general, the redox couples NAD/NADH and NADP/NADPH play a critical role in energy metabolism, glycolysis, tricarboxylic acid cycle and mitochondrial respiration. The increased NAD(P)H level in cells is linked to the abnormal production of reactive oxygen species (ROS) and DNA damage. However, due to the lack of sensitive NAD(P)H probe, it has been challenging to detect intracellular NAD(P)H in biological systems. Cell Meter™ Intracellular NADH/NADPH Fluorescence Imaging Kit provides an efficient method to monitor intracellular NAD(P)H level in live cells. JZL1707 NAD(P)H sensor has been developed as an excellent fluorescent probe for detecting and imaging NADH/NADPH in cells. The probe bind NADH/NADPH to generate strong fluorescence signal with high sensitivity and specificity. JZL1707 NAD(P)H sensor can be readily loaded into live cells, and its fluorescence signal can be conveniently monitored using the filter set of Cy3® or TRITC. This kit is optimized for fluorescence imaging and microplate reader applications.

AT A GLANCE

Protocol summary

1. Prepare cells in growth medium
2. Incubate cells with test compounds and JZL1707 NAD(P)H Sensor working solution at 37°C 30 - 60 minutes
3. Wash and keep cells in Assay Buffer
4. Monitor fluorescence intensity (bottom read mode) at Ex/Em = 540/590 nm (Cutoff = 570 nm) or fluorescence microscope with TRITC filter

Important
Thaw all the kit components at room temperature before starting the experiment.

KEY PARAMETERS

Instrument:	Fluorescence microplate reader
Excitation:	540 nm
Emission:	590 nm
Cutoff:	570 nm
Recommended plate:	Black wall/clear bottom
Instrument specification(s):	Bottom read mode
Instrument:	Fluorescence microscope
Excitation:	TRITC filter
Emission:	TRITC filter
Recommended plate:	Black wall/clear bottom

PREPARATION OF WORKING SOLUTION

Add 10 µL of JZL1707 NAD(P)H Sensor stock solution (Component A) into 2.5 mL of Assay Buffer (Component B), and mix well to make JZL1707 NAD(P)H Sensor working solution. This JZL1707 NAD(P)H Sensor working solution is stable within 1 hour at room temperature.

Note 40 µL of JZL1707 NAD(P)H Sensor stock solution is enough for one plate. Protect from light.

PREPARATION OF CELL SAMPLES

For guidelines on cell sample preparation, please visit
https://www.aatbio.com/resources/guides/cell-sample-preparation.html

SAMPLE EXPERIMENTAL PROTOCOL

1. To stimulate NADP/NADPH, treat cells with 10 µL of 10X test compounds (96-well plate) or 5 µL of 5X test compounds (384-well plate) in serum free medium or your desired buffer (such as PBS or HHBS). For control wells (untreated cells), add the corresponding amount of medium or compound buffer.

   Note JZL1707 NAD(P)H Sensor is serum sensitive, therefore it's recommended to keep cells in serum-free medium or the buffer of your choice. Alternatively, cells can be prepared and treated in regular full medium. Change to serumfree medium or buffer of your choice when incubation with JZL1707 NAD(P)H Sensor.

2. Add 100 µL/well (96-well plate) or 25µL/well (384-well plate) of JZL1707 NAD(P)H Sensor working solution in the cell plate. Co-incubate cells with test compound and JZL1707 NAD(P)H Sensor working solution at 37°C for 30-60 minutes, protected from light.

   Note For a NADH/NADPH positive control treatment: HeLa cells were incubated with 100 µM NADH or NADPH for 30 minutes in serum-free medium, and co-incubated with JZL1707 NAD(P)H sensor working solution at 37°C for another 30 minutes. See Figure 1 for details.

3. Wash cells with your desired buffer once. Remove solution in each well and add Assay Buffer (Component B) 100 µL/well for a 96-well plate or 25 µL/well for a 384-well plate.

4. Monitor the fluorescence increase using microplate reader at Ex/Em = 540/590 nm (Cutoff = 570 nm) with bottom read mode, OR take images using fluorescence microscope with the filter set of Cy3® filter or TRITC.

EXAMPLE DATA ANALYSIS AND FIGURES

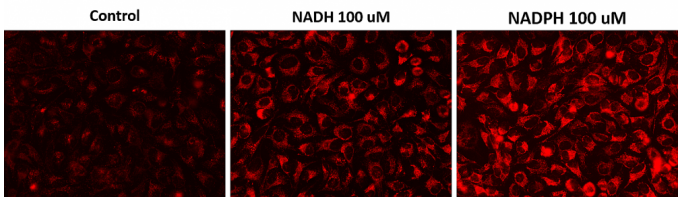

Figure 1.

Fluorescence images of NADH/NADPH in HeLa cells using Cell Meter™ Intracellular NADH/NADPH Fluorescence Imaging Kit (Cat#15290). HeLa cells were incubated with 100 µM NADH or 100 µM NADPH in serum-free medium for 30 minutes and then co-incubated with JZL1707 NAD(P)H sensor working solution for another 30 minutes. The fluorescence signal was measured using fluorescence microscope with a Cy3® filter.

DISCLAIMER

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